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Analysis of Asn-linked glycans from vegetable foodstuffs: widespread occurrence of Lewis a, core α1,3-linked fucose and xylose substitutions

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The N-glycans from 27 "plant" foodstuffs, including one from a gymnospermic plant and one from a fungus, were prepared by a new procedure and examined by means of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). For several samples, glycan structures were additionally investigated by size-fractionation and reverse-phase high-performance liquid chromatography in conjunction with exoglycosidase digests and finally also ^{1}H-nuclear magnetic resonance spectroscopy. The glycans found ranged from the typical vacuolar "horseradish peroxidase" type and oligomannose to complex Le^a-carrying structures. Though the common mushroom exclusively contained N-glycans of the oligomannosidic type, all plant foods contained mixtures of the above-mentioned types. Apple, asparagus, avocado, banana, carrot, celery, hazelnut, kiwi, onion, orange, pear, pignoli, strawberry, and walnut were particularly rich in Le^a-carrying N-glycans. Although traces of Le^a-containing structures were also present in almond, pistachio, potato, and tomato, no such glycans could be found in cauliflower. Coconut exhibited almost exclusively N-glycans containing only xylose but no fucose. Oligomannosidic N-glycans dominated in buckwheat and especially in the legume seeds mung bean, pea, peanut, and soybean. Papaya presented a unique set of hybrid type structures partially containing the Le^a determinant.

These results are not only compatible with the hypothesis that the carbohydrate structures are another potential source of immunological cross-reaction between different plant allergens, but they also demonstrate that the Le^a-type structure is very widespread among plants.

Key words: allergy/MALDI-TOF-MS/Lewis a/plant glycoproteins

Introduction

Food allergies are major health problems, and there are many descriptions in the literature of either anaphylaxis or oral allergy to foods, contact dermatitis, asthma induced by food particles (through, e.g., exposure in the food processing industry), or positive skin tests in patients allergic to foods or pollens. Often patients are hypersensitive and/or have positive skin tests to a certain group of foods or other plant extracts, including pollens (e.g., birch-nut-fruit, mugwort-celery-carrot-spice and latex-fruit syndromes) (Helbling, 1997); the presence of cross-reactive IgE, though, does not always correlate with symptoms or positive skin tests, thus leading to false-positive results in radioallergosorbent tests (RASTs) (van Ree and Aalberse, 1999).

Food glycans are of interest because the IgE cross-reactions that have been reported between pollen and vegetable foods are at least partly due to carbohydrate components (Aalberse and van Ree, 1997; van Ree *et al.*, 2000); indeed it has been claimed that major food allergens are typically water-soluble glycoproteins (Bernhisel-Broadbent, 1995). The roles of proteins themselves in cross-reactivity should, however, not be understated because many clinically relevant cross-reactions are between proteins (Breiteneder, 1998), such as analogues of Bet v I (Schöning *et al.*, 1995), profilins (e.g., Bet v II) (Valenta *et al.*, 1992), isoflavone reductases (Vieths *et al.*, 1998), and hevein/type I chitinases (Salcedo *et al.*, 1999).

Carbohydrate-dependent cross-reactions have been probed by crude means, such as examining loss of binding of IgE to periodate-oxidized or chemically deglycosylated food extracts. More specifically, neoglycoconjugates consisting of glycopeptides carrying core α1,3-linked fucose coupled to bovine serum albumin have been found, as judged by direct enzyme-linked immunoassay (ELISA) and/or inhibition ELISA, to bind IgE from either pollinosis patients who displayed also food hypersensitivity or patients allergic to celery (Petersen *et al.*, 1997; Fötisch *et al.*, 1999). In addition, studies in this laboratory have shown that IgG antibodies recognizing core α1,3-linked fucose will bind a range of pollen and food extracts (Wilson *et al.*, 1998); indeed a structural survey of glycans verified the presence of this epitope in 10 pollens (Wilson and Altmann, 1998). Whether such carbohydrate-mediated cross-reactions are clinically significant is controversial (Van der Veen *et al.*, 1997), although core α1,3-fucosylated glycoconjugates have been shown to elicit the release of histamine from mast cells (Batanero *et al.*, 1999; Fötisch *et al.*, 1999), suggesting that glycans may indeed be biologically active in allergy. However, it is obvious that their cross-reactivity can complicate allergy diagnosis, and this assumption is corroborated in several reports (van Ree and Aalberse, 1993,

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1999; Aalberse, 1997; Aalberse and van Ree, 1997; Mari *et al.*, 1999; van Ree *et al.*, 2000).

Among cross-reactions of interest in the present study, an allergy to latex is often associated with hypersensitivity to avocado, banana, and kiwi (M'Raihi *et al.*, 1991; Blanco *et al.*, 1994; Ahlroth *et al.*, 1995; Lavaud *et al.*, 1995; Möller *et al.*, 1998); in two cases, cross-reactivity between latex and buckwheat was also reported (de Maat-Bleeker and Stapel, 1998). On the basis of comparing IgE blots with Western blotting with an anticarbohydrate antibody, these cross-reactions to latex have recently been assigned to chitinases, rather than to N-linked oligosaccharides (Diaz-Perales *et al.*, 1999). On the other hand, possibly complicating the picture also is the finding that kiwi allergy can also be associated with birch allergy (Gall *et al.*, 1994); one study suggested that this cross-reaction is mainly due to carbohydrate (Fahlbusch *et al.*, 1998). A similar conclusion was made about the cross-reaction between latex and grass pollen (Fuchs *et al.*, 1997). Indeed, both birch and grass pollens carry N-glycans with xylose and core α1,3-linked fucose (Wilson and Altmann, 1998). In yet another example of overlapping allergy syndromes, sensitivity to birch or mugwort pollen can also be associated with allergy to apple and other Rosaceae fruits, carrot, celery, and potato (Calkhoven *et al.*, 1987; Ebner *et al.*, 1996; Heiss *et al.*, 1996; Fernández-Rivas *et al.*, 1997). Of specific interest with regard to the present study, RAST of apple- and peach-allergic patients can be inhibited up to 85% by proteinase K–digested pollen extract, suggestive of the importance of nonprotein (possibly carbohydrate) components in the cross-reactivity (van Ree *et al.*, 1995); in another study, a number of celery-allergic patients displayed carbohydrate cross-reactivity as judged by periodate oxidation and by binding to a neoglycoconjugate containing the MUXF[3] structure (Jankiewicz *et al.*, 1998).

Hazelnut allergy has also been described in tree pollen–allergic patients (Hirschwehr *et al.*, 1992). Indeed, nuts, whether legume peanut or true tree nuts, are a major source of allergy (Burks *et al.*, 1998; Sicherer *et al.*, 1999); a rare allergy to coconut was also found to be associated with allergy to other nuts, especially walnut, but also almond (Teuber and Peterson, 1999). Among other foods examined in the present study, allergic reactions to asparagus (Eng *et al.*, 1996; Escribano *et al.*, 1998), cauliflower (van Ketel, 1975), pignoli (pine nut) (de las Marinas *et al.*, 1998), onion (Valdivieso *et al.*, 1994), pistachio (Parra *et al.*, 1993; Fernandez *et al.*, 1995; Liccardi *et al.*, 1996), strawberry (Grattan and Harman, 1985), and tomato (Petersen *et al.*, 1997) have also been reported.

As part of ongoing studies in this laboratory to verify the structural basis for carbohydrate-mediated IgE cross-reactivity, the glycan structures from twenty-seven food extracts have been examined. By means of matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS), supplemented by size-fractionation and reverse-phase high-performance liquid chromatography (RP-HPLC) as well as nuclear magnetic resonance (NMR) spectroscopy, it was found that all plant foods contain N-glycans carrying xylose and core α1,3-linked fucose, and many also contain Le^a epitopes; thus, not even considering O-glycans or cell wall polysaccharides, it is obvious that N-glycans are major candidates for explaining carbohydrate cross-reactivity between different plant species.

Results

In this study, the N-glycans from 26 plant foods and 1 mushroom, a foodstuff erroneously regarded as a plant by the average consumer, were analyzed. The principal method for N-glycan analysis was MALDI-TOF-MS. Various N-glycans from several plant foods were subject to additional experiments, such as HPLC of pyridylaminated glycans, exoglycosidase digestion, and, in the case of strawberry, NMR spectroscopy. These results are valuable additions to the mass data and will be explained in some detail below.

MALDI-TOF-MS profiling of N-linked oligosaccharides from plant foods

The Asn-linked glycans from various foodstuffs, covering large sections of the phylogenetic tree of plants, were analyzed by MALDI-TOF-MS of underivatized oligosaccharides prepared by immediate proteolytic digestion of these foods. This procedure has been devised to avoid two possible errors inherent to the analysis of soluble extracts as has been performed previously (Wilson and Altmann, 1998). First, the immediate pepsin digestion of proteins at pH 2 is considered to prevent any enzymatic degradation of glycans that might occur during extraction. Second, N-glycans from insoluble, e.g., membrane-associated, glycoproteins will likewise be included by this technique. Due to the different masses of hexoses, GlcNAc, xylose, and fucose, MS allows a fairly detailed insight into the structural species present in a sample. Assuming a conserved biosynthetic pathway of N-glycans in plants (Lerouge *et al.*, 1998), masses can be translated to primary structures as depicted in Figure 1. Several examples of N-glycan profiles obtained by this procedure are shown in Figure 2, and Table I gives a comprehensive overview of the results of our study. As will be shown below, in several cases the conclusions drawn from mass data were proven valid by other methods. The purification procedure relies on the affinity of peptides and glycopeptides to cation exchange resin under fairly acidic conditions. Even under these conditions, a small percentage of very acidic peptides or peptides with very acidic glycan moieties may not bind. The latter, however, are not supposed to occur in plant glycoproteins (Lerouge *et al.*, 1998). After release of the (neutral) oligosaccharide from peptide, the glycans will no longer bind to the resin. Additional purification steps, such as gel filtration and reversed-phase chromatography, facilitated the preparation of N-glycans from whole foodstuffs in sufficient purity to be analysed by MALDI-TOF-MS.

The relative amounts of individual structures in each sample was calculated from peak areas without any correction. As the N-glycan profiles extend from approximately 1000 to over 2000 Da, a mass bias appears possible. However, where available (e.g., for apple, banana, celery, kiwi, and strawberry), the quantitation based on sizing HPLC of pyridylaminated glycans gave comparable results. Thus, we assume the quantitations by MALDI-TOF to be a fair reflection of the true composition of the mixtures. Indeed, to ascertain the reliability of the determinations of the percentages, the results from 16 of the samples were subjected to statistical analysis: three spectra were recorded from individual spots of each of these samples. For peaks accounting for at least 5% of the total glycans in a sample, the relative standard deviations (i.e., standard deviation

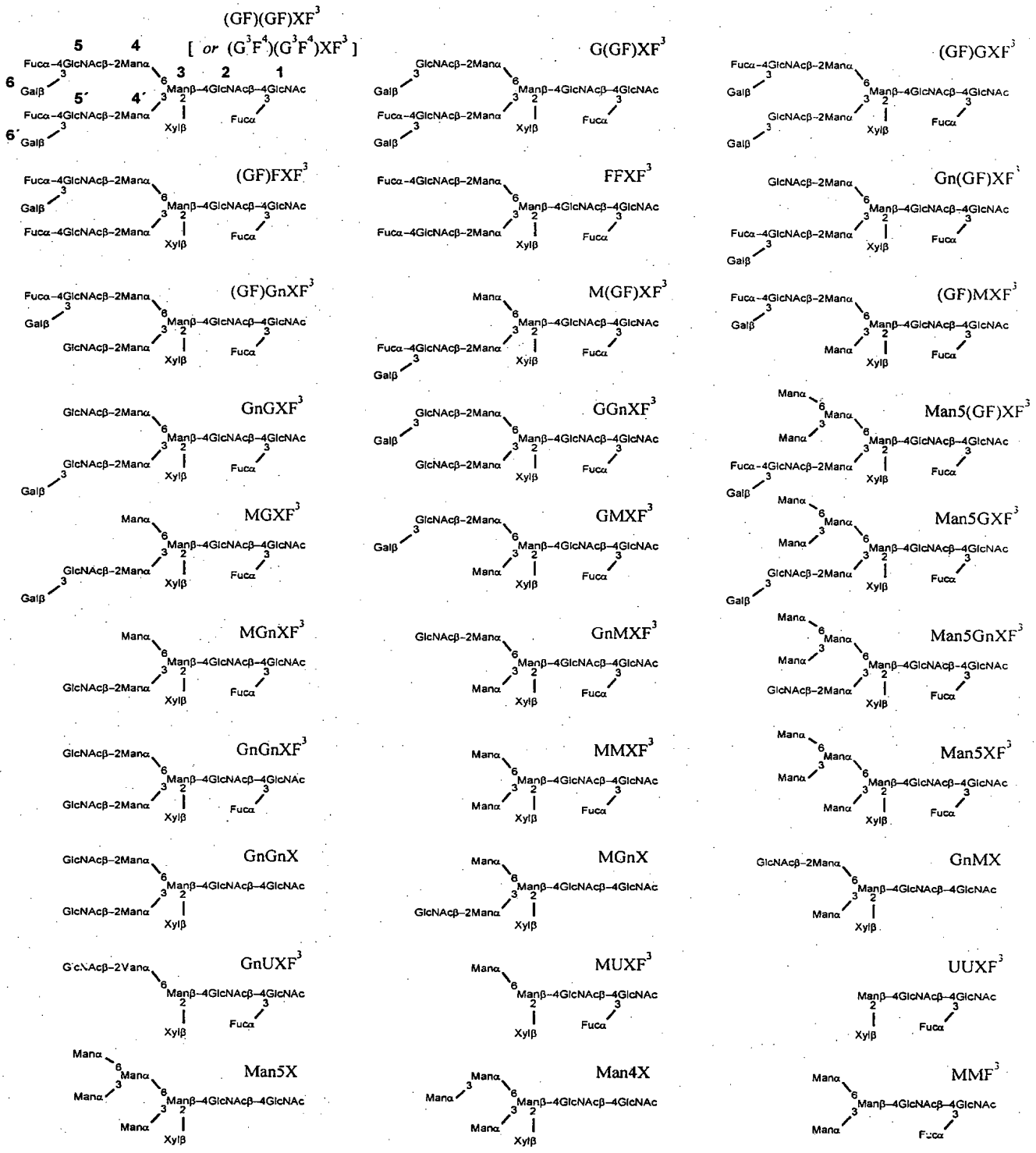

Fig. 1. Structures of complex-type N-glycans found in vegetable foods. The abbreviation system applied herein names the terminal residues starting with the residue on the 6-linked antenna and proceeding counterclockwise whereby Gal = G; GlcNAc = Gn, Xyl = X, Fuc = F, and Man = M. Where two terminal residues are located on one arm, as in the case of the Le^a determinant, they are enclosed in brackets. In case of residues that may occur in more than one type of linkage—as, e.g., core fucose (α1,3- or α1,-6), galactose (β1,3 or β1,4,) or outer arm fucose (α1,3- or α1,4)—the linkage of the sugar may be depicted as a superscript. However, where defined and clear from the context, these superscripts may also be omitted, as has herein been done in the case of the Le^a-antennae.

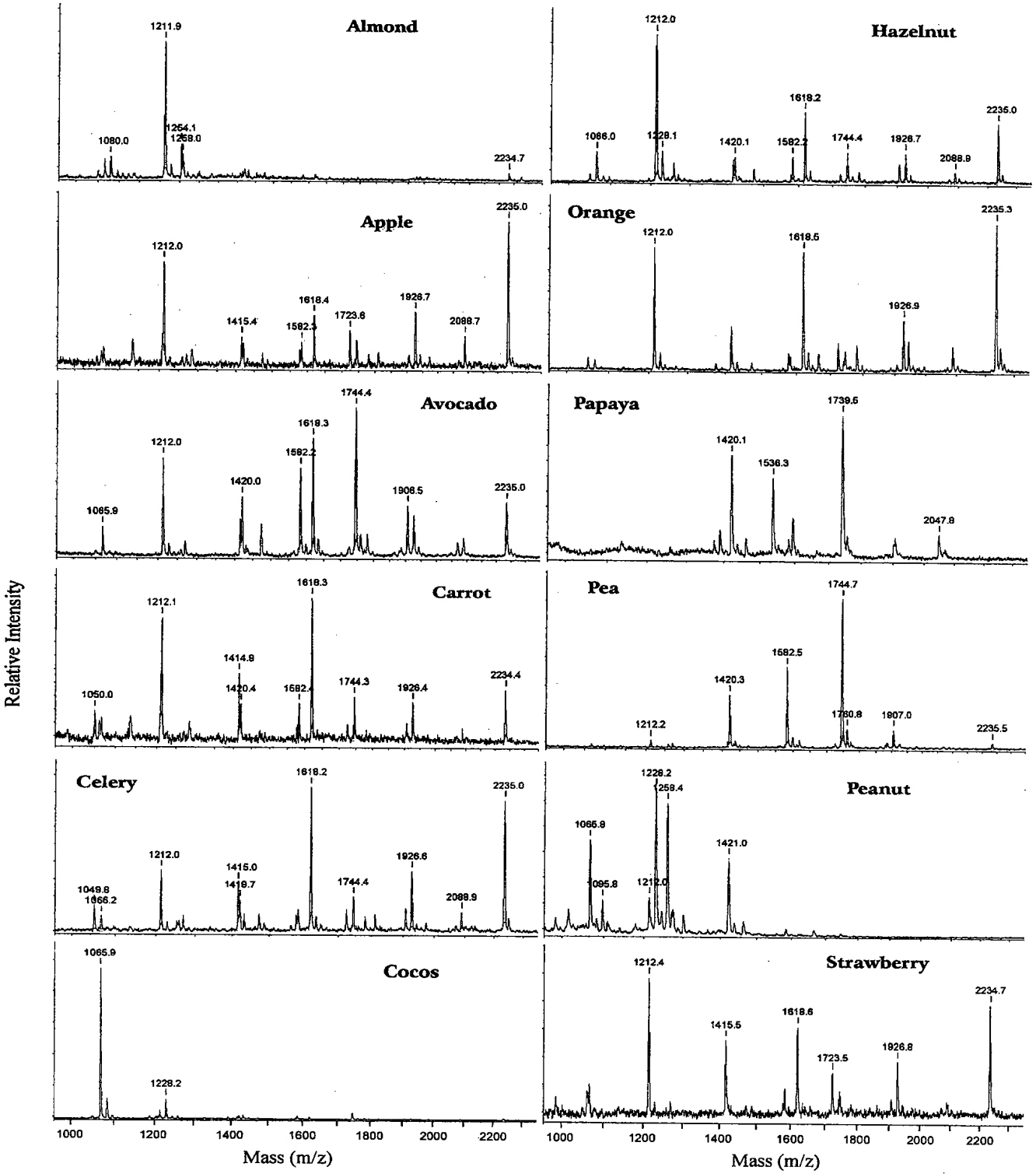

Fig. 2. Mass-spectrometric glycome profiles of selected food crops. Only significant peaks are identified by their mass ($M+Na^+$). A summary of all masses and the respective structures is given in Table I.

calculated as a percentage of the abundance) were up to ±25% in 73 and up to ±10% in 39 of 76 cases. In extreme cases, peaks at or below the 1% level were occasionally not detectable in one or two of three spectra obtained.

Variation of the quantitative pattern can also arise from the samples themselves as the N-glycan profile may depend on variety, ripening status, and storage conditions. For instance, in sequential preparations of peanut glycans (GF)(GF)XF^3 was detected (albeit as a minor component) in very fresh peanuts, whereas this structure was no longer detectable after 10 weeks of storage at room temperature. Generally, however, the results from those foods from which two or more preparations had been performed (almond, apple, celery, coconut, pea, peanut, pear, potato, tomato, and strawberry) clearly showed that the overall profile remains conserved: the major species in one preparation are the major species in a duplicate preparation.

Artifactual variation can be introduced by inappropiate methodology, for instance, by lengthy extraction procedures. For example, when analyzed by the direct proteolysis method MMX accounted for only 6% of hazelnut glycans, whereas in the soluble extract this was a main compound and the relative amount of larger glycans was reduced. Likewise, tomato N-glycans when analyzed from extracts of acetone powder (Zeleny *et al.*, 1999) contained relatively much more MUXF^3 and other very small structures than the directly proteolyzed sample (compare Table IV in Zeleny *et al.*, 1999; and Table I herein). Therefore, we believe that the direct proteolysis method used in the current study results in a less distorted and more accurate overview of a sample's N-glycan profile than any other recently applied procedure.

HPLC analysis of glycans

To demonstrate the validity of the mass spectrometric analyses, structures of the major N-glycans in some of the samples were elucidated by additional methods. In an approach similar to our previous study on pollens (Wilson and Altmann, 1998) and tomato (Zeleny *et al.*, 1999), pyridylaminated N-linked oligosaccharides from proteolyzed slurries of apple, banana, celery, kiwi, and strawberry and from soluble extracts of almond, avocado, coconut, pea, pistachio, and soya were fractionated according to size as shown for celery in Figure 3. Each nonvoid peak collected from size fractionation HPLC was rechromatographed by RP-HPLC; in addition, the full spectrum of N-glycans was also analysed by RP-HPLC of pyridylaminated N-glycans (data not shown). The effects of exoglycosidase digestion on retention times were compared with the properties of standard oligosaccharides MMXF^3, MUXF^3, MMX, and MM to assign the structure. In a number of cases, fractions were subject to MALDI-TOF-MS analysis. For kiwi and banana N-glycans identity was inferred by comparison of their three-dimensional properties to those of corresponding celery glycans, but additional exoglycosidase digests were not performed. In the following account of experimental evidence results are presented in groups of related structures. As a detailed rationale for the structural assignments of N-glycans of the oligomannosidic and of the truncated type (up to GnGnXF^3) has been presented in previous articles (Kubelka *et al.*, 1994; Wilson and Altmann, 1998), the respective results for food N-glycans will only be outlined here.

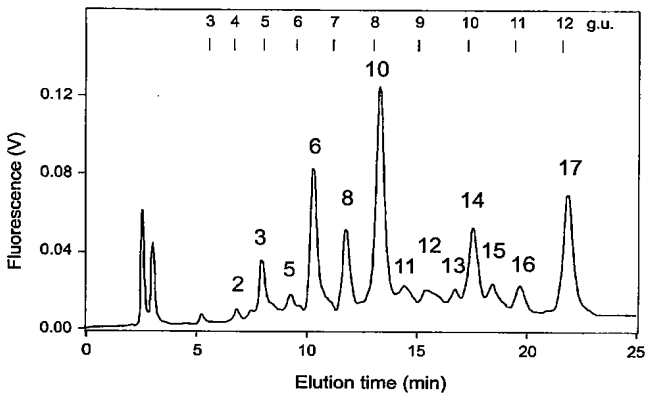

Fig. 3. Elution profile of pyridylaminated celery N-glycans on the Micropak sizing column. Individual peaks were further fractionated by RP-HPLC and subject to various exoglycosidase treatments as described in the text

Solely xylosylated structures (MMX, Man4X, GnGnX). By HPLC retention times (5.6 glucose units by Micropak = 5.6 g.u.-MP and 7.7 g.u. by RP-HPLC = 7.7 g.u.-RP) and comparison with the effect of digestion of the standard MMX (first to 11 and then 8.9 g.u.-RP after α-mannosidase), MMX was found to be the major component of the coconut glycome ([M+Na]$^+$ = 1144.2), as well as being present in almond ([M+Na]$^+$ = 1143.5), avocado, celery ([M+H]$^+$ = 1123.4), soybean, and strawberry. Man4X was identified in coconut ([M+Na]$^+$ = 1306.5) due to its size (6.2 g.u.-MP), and its α-mannosidase sensitivity was similar to that of MMX with digestion products of 8.5 and 10.5 g.u, but its initial retention time by RP-HPLC is slightly greater. Considering the digestion pathway of Man5 and Man4 by jack bean α-mannosidase (Tomiya *et al.*, 1991) and the large effect of mannosidase on Man4X, we assume the initial removal of the mannose residue in 3-linkage to the α-mannose (Wilson and Altmann, 1998). As the intermediate Manα–3Manα–6(Xylβ–2)Manβ–4GlcNAcβ–4GlcNAc (M^{3}UX) had a different RP retention time than the presumed MMX (see Figure 1 for structure), the data on Man4X also give credence to the structural assignment of MMX. GnGnX was identified in apple and celery ([M+Na]$^+$ = 1550.1; 6.9 g.u.-MP; 9.9 g.u.-RP), which is comparable to the value for GnGnX found in olive pollen (Wilson and Altmann, 1998).

Truncated structures with both xylose and fucose (UUXF^3, MUXF^3, MMXF^3, GnMXF^3, MGnXF^3, GnGnXF^3). UUXF^3 was identified in apple and celery ([M+Na]$^+$ = 967.2) based on its RP-HPLC retention time (5.2 g.u.-RP) being identical to the final mannosidase digest product of MUXF^3 and MMXF^3. According to "three-dimensional" properties, MUXF^3 was found in apple, avocado, celery ([M+Na]$^+$ = 1128.3), and soybean extract and MMXF^3 in almond ([M+Na]$^+$ = 1290.4), apple, avocado, celery ([M+H]$^+$ = 1268.6), coconut ([M+Na]$^+$ = 1290.7), pea, pistachio ([M+Na]$^+$ = 1291.6), soybean, and strawberry ([M+H]$^+$ = 1268.2). α-Mannosidase digestions of these glycans resulted in the expected shift to UUXF^3 (5.2 g.u.-RP). In the case of MMXF^3 an intermediate digestion product of 6.0 g.u. on reverse-phase (the MUXF^3 structure) was apparent because the α1,6-mannose is more resistant to α-mannosidase treatment. On further incubation with α-mannosidase, this intermediate product was converted completely or almost completely to UUXF^3 in all cases.

GnMXF^3 and MGnXF^3, as present in almond, apple, avocado, celery ([M+Na]$^+$ = 1492.6), pea, and strawberry

Table I. N-glycan profiles of edible parts of food crops

m/z (M+Na⁺)	Structure	Almond	Apple	Asparagus	Avocado	Banana	Buckwheat	Carrot	Cauliflower	Celery	Coconut	Hazelnut	Mung bean	Mushroom	Kiwi	Onion	Orange	Papaya	Pea	Peanut	Pear	Pignoli	Pistachio	Potato	Soya	Strawberry	Tomato	Walnut
Oligomannosidic structures																												
1096,0	Man4	1.8	1.0	—	—	—	—	—	—	—	—	—	0.5	—	—	—	—	—	—	3.9	—	—	—	—	0.2	—	—	—
1258.4	Man5	10.8	0.9	—	0.4	—	1.8	—	1.2	0.7	1.1	2.8	2.7	9.9	1.9	—	—	0.6	0.6	27.5	—	22.3	8.7	—	1.3	—	—	3.7
1420.2	Man6	1.3	3.0	0.7	7.0	5.7	6.2	5.7	5.1	4.0	1.5	4.4	9.3	11.3	0.6	1.2	0.4	22.3	14.8	23.1	3.6	0.3	0.3	—	11.2	—	3.5	3.9
1582.4	Man7	1.0	2.7	1.9	10.4	2.8	14.0	6.5	4.5	2.4	1.5	4.7	31.1	38.4	3.1	1.8	1.8	1.3	23.8	1.4	2.6	0.6	2.4	2.7	23.6	2.6	3.6	6.2
1744.5	Man8	1.4	3.9	7.3	18.5	4.3	11.0	6.1	8.0	4.5	2.6	4.9	42.2	33.8	4.6	2.6	2.3	—	48.2	0.3	3.2	2.5	1.9	2.1	56.0	3.9	5.3	8.1
1907.1	Man9	2.0	2.1	2.3	6.6	5.3	20.3	2.1	3.8	3.2	—	3.1	11.2	5.1	1.3	1.6	0.6	1.6	4.5	—	3.7	—	0.5	1.0	2.4	2.3	2.2	0.6
2068.8	Man9Glc1	—	0.8	1.2	2.0	0.4	0.4	0.4	1.3	—	—	—	0.2	1.5	—	0.6	0.3	—	0.1	—	0.7	—	—	—	—	0.5	0.4	—
	Sum	18.3	13.4	13.4	44.9	18.6	53.7	20.8	23.9	14.8	6.7	19.9	97.2	100	11.6	7.8	5.4	25.8	92.0	56.2	13.8	25.7	13.8	5.8	94.6	9.4	15.0	22.5
Complex-type structures with only Fuc																												
1080.0	MMF	8.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.8
Complex-type structures with only Xyl																												
903.8	MUX	—	—	—	—	0.1	—	—	—	—	—	0.2	—	—	0.9	—	—	—	—	—	—	—	—	—	—	—	—	—
1065.7	MMX	7.3	1.3	0.9	2.8	0.2	1.2	2.3	3.9	2.2	76.1	6.0	—	—	7.0	4.7	0.4	—	0.3	8.9	2.5	—	9.1	3.5	0.3	2.9	6.8	5.3
1228.1	Man4X	—	—	—	0.4	—	—	16.4	—	—	9.6	—	—	—	1.2	—	—	0.4	—	29.9	—	—	—	—	—	—	0.6	—
1269.1	GnMX / MGnX	0.2	1.3	1.6	1.3	1.7	4.0	—	1.3	1.2	—	0.6	0.1	—	3.1	0.7	—	—	0.8	1.5	3.8	—	—	0.2	—	2.1	1.6	1.4
1431.3	Man4GnX	1.9	—	—	0.4	—	0.8	—	—	0.9	2.0	—	—	—	—	—	—	—	0.2	—	—	—	0.2	—	—	—	—	0.7
1472.1	GnGnX	—	1.3	0.8	3.5	2.9	2.5	—	5.0	2.3	—	4.6	—	—	2.6	1.7	0.5	—	0.2	—	2.7	—	—	—	—	1.1	3.0	1.0
	Sum	9.4	3.9	3.3	8.4	4.9	8.5	18.7	10.2	6.6	87.7	11.4	0.1	0.0	14.8	7.1	0.9	0.4	1.5	40.3	9.0	0.0	9.3	3.7	0.3	6.1	12.1	8.4
Complex-type structures with Fuc and Xyl																												
887.8	UUXF	—	—	—	—	—	—	—	—	—	—	0.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1049.7	MUXF	1.2	1.1	1.4	0.7	4.2	0.2	3.8	—	3.4	—	0.9	—	—	1.6	8.7	1.1	—	—	—	0.1	—	3.2	—	—	0.9	5.0	1.2
1212.1	MMXF	57.4	14.1	15.0	10.4	3.2	16.8	—	17.1	8.5	3.4	31.8	1.2	—	8.1	9.5	17.7	—	2.0	2.5	13.8	25.8	58.2	76.7	3.3	22.4	31.2	32.8
1253.1	GnUXF	—	—	—	0.3	0.6	—	—	—	0.4	—	—	—	—	0.1	0.4	—	—	—	—	—	—	—	—	—	—	—	2.8
1374.2	Man4XF	—	1.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.9	—	—	—	—	1.0	—	—	0.3	—	0.7
1415.5	GnMXF / MGnXF	1.8	4.5	8.5	3.9	6.5	8.1	8.8	7.7	6.4	0.8	3.2	—	—	4.0	10.0	5.6	—	0.9	—	7.4	5.1	1.8	3.9	—	11.9	14.8	4.2
1577.4	GMXF / MGXF	—	1.8	1.8	0.2	2.2	—	3.1	0.7	1.3	—	1.0	—	—	1.4	0.8	2.3	—	—	—	1.5	—	—	—	—	0.7	—	1.3
1618.5	GnGnXF	1.3	8.4	29.0	14.6	20.5	7.7	25.3	40.1	21.9	1.0	12.3	0.7	—	13.5	25.5	19.1	—	1.7	—	17.8	17.6	3.9	6.3	0.6	13.4	17.8	3.5
1780.4	GGnXF / GnGXF	—	1.0	3.5	2.0	2.4	—	1.2	—	1.2	—	1.5	—	—	1.8	1.2	4.0	—	—	—	2.3	—	—	—	—	0.4	0.7	0.9
	Sum	58.7	29.1	53.4	29.9	34.9	32.8	39.1	64.9	40.6	5.2	48.4	1.9	0.0	27.2	56.1	43.5	0.0	4.6	2.5	39.2	48.5	67.1	86.9	3.9	50.0	70.0	44.3
Complex type structures with Lewis A determinants																												
1723.7	(GF)MXF / M(GF)XF	—	6.9	5.2	—	4.9	0.4	2.0	—	2.1	—	1.3	—	—	3.6	5.9	3.8	—	—	—	2.0	2.0	0.8	—	—	7.3	0.5	2.7
1926.7	(GF)GnXF / Gn(GF)XF	1.0	10.4	7.2	4.3	14.6	1.0	5.7	—	9.0	—	4.6	—	—	12.3	8.2	8.4	—	0.4	—	8.4	8.8	2.0	1.6	0.1	9.0	1.0	3.2
2088.8	(GF)GXF / G(GF)XF	—	5.2	1.4	2.1	—	0.4	2.0	—	2.6	—	1.6	—	—	3.3	1.2	3.7	—	0.1	—	3.5	0.6	0.9	—	—	0.8	—	3.9
2235.0	(GF)(GF)XF	4.0	28.2	11.0	8.2	17.4	3.1	8.5	—	21.9	0.5	10.4	0.7	—	24.0	13.7	27.9	—	1.1	0.9	20.4	14.3	4.7	2.0	1.1	17.4	1.4	9.4
	Sum	5.0	50.7	24.7	14.6	36.9	4.9	18.2	0.0	35.6	0.5	17.9	0.7	0.0	43.2	29,0	43.8	0.0	1.6	0.9	34.2	25.8	8.4	3.6	1.2	34.5	2.9	19.2
Substituted pentamannosidic structures																												
1390.2	Man5X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3.3	—	—	—	—	—	—	—	—	—	—
1461.3	Man5Gn	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.6	—	—	—	—	—	—	—	—	—	—
1536.4	Man5XF	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	18.7	—	—	—	—	0.3	—	—	—	—	—
1593.4	Man5GnX	—	—	—	—	—	—	—	0.4	—	—	—	—	—	—	—	—	7.1	—	—	—	—	—	—	—	—	—	—
1739.6	Man5GnXF	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	38.8	—	—	—	—	—	—	—	—	—	—
1901.7	Man5GXF	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.2	—	—	—	—	—	—	—	—	—	—
2047.8	Man5(GF)XF	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.2	—	—	—	—	—	—	—	—	—	—

The N-linked oligosaccharides released by N-glycosidase A were analyzed by MALDI-TOF-MS. The structures of N-glycans are depicted in Figure 1. According to current knowledge and HPLC data from this work, the core fucose is α1,3-linked, hence the full abbreviation of MMXF would be $MMXF^3$. The terminal Gal and Fuc residues are generally assumed to form the Le^a-determinant as proven for strawberry and celery. Hence, (GF)MXF could also be written as $(G^3F^4)MXF^3$ to fully specify the linkages. The abundances of structures are given in percentages and were deduced from peak areas from representative spectra.

([M+Na]$^+$ = 1493.1), co-elute on Micropak (7.3 g.u.) but separate on RP-HPLC (5.8 and 4.7 g.u.). Similar to the previously reported results with pollen N-glycans (Wilson and Altmann, 1998), GnMXF^3 (from almond and pea) was sensitive to α-mannosidase as shown by its conversion to GnUXF^3 eluting at 8.0 g.u.-RP. On the other hand, MGnXF^3 is apparently insensitive to this treatment, compatible with the known resistance of α1,6-linked mannose (Oku *et al.*, 1990). GnUXF^3 itself was found in celery due to its retention time of 7.8 g.u.-RP and its mass ([M+Na]$^+$ = 1330.1).

GnGnXF^3 (8.2 g.u.-MP, 5.4 g.u.-RP) was found in apple, celery ([M+Na]$^+$ = 1696.5), and strawberry ([M+Na]$^+$ = 1698.9). β-Hexosaminidase treatment of GnMXF^3, MGnXF^3, or GnGnXF^3 resulted in a single peak of 4.8 g.u.-RP, which is the retention expected for MMXF^3. Subsequent α-mannosidase digestion, when performed, of hexosaminidase-treated fractions showed the same pattern of digestion indicative of the MMXF^3 structure.

In addition to these experiments, celery fractions assigned as MMXF^3 and GnGnXF^3 were subject to monosaccharide analysis by gas liquid chromatography–mass spectrometry (GLC-MS) and found to contain besides fucose, mannose and GlcNAc only xylose but no arabinose.

Solely fucosylated structures (MMF3). MMF^3 was identified in almond ([M+Na]$^+$ = 1158.2) due to its α-mannosidase sensitivity (shift from 5.0 to 4.5 g.u.-RP) being similar to that of MMF^3 from bee venom phospholipase (Kubelka *et al.*, 1993).

Galactosylated structures with xylose and core α1,3-linked fucose. Two structures only resolvable by RP-HPLC but consistent in terms of mass with being GnGXF^3 and GGnXF^3 were found to be present in celery fraction 12 (12c and 12d). Treatment of 12c with β-hexosaminidase resulted in a shift in RP-HPLC elution time from 5.2 to 4.7 g.u, whereas treatment of 12d with β-hexosaminidase resulted in a shift from 5.4 to 6.0 g.u.-RP. This product was purified and then subjected to a combined mannosidase and galactosidase treatment, resulting in a peak of 7.8 g.u.-RP. Thus, it is concluded that fraction 12d contains GGnXF^3 because the combined enzyme digestions resulted in an increase in elution time to the same as the elution time of GnUXF^3, whereas the decrease in elution time on hexosaminidase treatment of 12c would suggest that the galactose was on the 3-arm (i.e., a structure of GnGXF^3).

HPLC analysis of structures containing the Le^a epitope. Oligosaccharides with a long retention time on the sizing column (8–12 g.u.-MP) were found in apple, banana, celery, kiwi, and strawberry, but which by RP-HPLC had similar retention times in the range 4.8–5.8. g.u., thus making them and their exoglycosidase digestion products difficult to distinguish by the latter HPLC method. However, on the basis of mass, the compositions of these glycans are compatible with the presence of terminal galactose and fucose residues, in addition to core xylose and α1,3-linked fucose. Bearing in mind the recent findings on the presence of Le^a epitopes in plants, putative Le^a-containing structures from celery and strawberry were analyzed by HPLC in conjunction with exoglycosidase digests as follows.

The largest N-glycan, e.g., from celery (peak 17 in Figure 3), had a mass of [M+Na]$^+$ = 2313.2, which is consistent with the complete secreted-type structure (Fitchette-Lainé *et al.*, 1997; Melo *et al.*, 1997). Its HPLC elution position (12 g.u.-MP, 5.2 g.u.-RP) was identical with the largest structure from sycamore cell laccase, which was previously shown to contain two Le^a-determinants (Fitchette-Lainé *et al.*, 1997). The putative (GF)(GF)XF^3 [or (G^{3}F^4)(G^{3}F^4)XF^3 if linkages are to be fully assigned] from celery as well as strawberry was sensitive to almond α-fucosidase, which removed two residues as judged by MicroPak and MALDI-TOF-MS. Partial fucosidase digest resulted in a mixture of monofucosylated products with elution times of 11.0 g.u.-MP and 5.0 and 5.2 g.u.-RP; full digestion gave a product of 10 g.u.-MP and 5.1 g.u.-RP. Because the core α1,3-fucose is not sensitive to almond α-fucosidase (Kubelka *et al.*, 1993), these data show that two outer-arm fucosyl residues had been removed. The defucosylated glycan now was sensitive to bovine kidney β-galactosidase but resistant to *Aspergillus oryzae* β-galactosidase. This was in contrast to the sensitivity of the β1,4-linked galactose residues of bovine fibrin glycans to the *Aspergillus* enzyme (data not shown). Since the fungal enzyme has a strong preference for β1,4-linkages (Zeleny *et al.*, 1997), the two glycans from celery and strawberry apparently contained β1,3-linked galactose residues. The structural analysis of strawberry (GF)(GF)XF^3 was completed by NMR spectroscopy (see *NMR spectrometric analysis of Le^a-containing structures*).

In addition to the complete vacuolar type, smaller species were found to be present in apple, celery, and strawberry when analysed by HPLC.

(GF)GXF^3/G(GF)XF^3 (celery 16; 11 g.u.-MP; 5.1–5.2 g.u.-RP; [M+Na]$^+$ = 2167) appeared to be a mixture of two isomers that had the chromatographic properties of a partial α-fucosidase digest of 17, which also results in two isomers (11.0 g.u.-MP; 5.1 and 5.2 g.u.-RP).

A small fraction of celery peak 16 behaved differently, having a RP-retention time of 5.9 g.u. and a mass of [M+Na]$^+$ = 2151. With α-fucosidase and β-hexosaminidase, there was no change in RP elution time of the presumed (GF)FXF^3, but the expected digestion product would also have 5.9 g.u.-RP (GMXF^3, cf. digestion of 12d). Subsequent digestion though with α-mannosidase resulted in a retention time of 7.0 g.u.-RP (presumably GUXF^3). Thus, we conclude this trace compound to have the structure (GF)FXF^3.

(GF)GnXF^3/Gn(GF)XF^3 (celery 14d; 10 g.u.-MP; 5.2 g.u.-RP; [M+Na]$^+$ = 2005) was insensitive to β-galactosidase but converted to a peak of 5.4 g.u. (RP-HPLC) on digestion with both almond α-fucosidase and β-galactosidase. β-hexosaminidase digestion resulted in the almost complete conversion of the doublet to two peaks (4.7 and 5.8 g.u.-RP), which correspond in retention time to M(GF)XF^3 and (GF)MXF^3, respectively, suggesting that celery peak 14d is a mixture of both possible isomers. The corresponding peak from strawberry was converted by almond α-fucosidase to a peak of 9.4 g.u.-MP and by combined fucosidase/galactosidase digestion to a peak of 8.2 g.u.-MP, suggestive of the respective removal of one and two residues. In theory, the Gal and Fuc residues might be located on different GlcNAc residues, but structures GFXF^3/FGXF^3 were present in only minute amounts, if at all, as judged from the β-hexosaminidase digest of celery 14d and from NMR in the case of the respective strawberry structure.

Celery peak 14 also contained a small amount of an additional structure (10.0 g.u.-MP; 4.8 g.u.-RP; $[M+Na]^+ = 1989$), tentatively assigned as $FFXF^3$ by means of its mass.

$M(GF)XF^3/(GF)MXF^3$ (celery 12b and 12e; 9.2 g.u.-MP; 4.8 and 5.8 g.u.-RP) were both insensitive to bovine testes β-galactosidase, but also a combined α-fucosidase and β-galactosidase digest of 12e resulted in no obvious change in elution time by RP-HPLC; previous studies have shown that the $GnMXF^3$ product of such a digest would have a retention time of 5.8. g.u.-RP anyway. However, a subsequent α-mannosidase digest resulted in $GnUXF^3$ as the probable final product (7.8 g.u.-RP), thus suggesting that 12e is indeed $(GF)MXF^3$, whereas the predominant isomer is presumably $M(GF)XF^3$.

For other samples, extensive fractionation and analysis of individual glycan species were not performed. However, the PA-glycans from apple, asparagus, buckwheat, carrot, cauliflower, hazelnut, onion, and pear were subject to RP-HPLC. The profiles obtained agreed with the assumption that these samples contained the same N-glycans as celery and strawberry.

NMR spectrometric analysis of Le^a-containing structures

Since the linkage of the fucose to the celery and strawberry putative Le^a-containing structures cannot be specified by the results of almond α-fucosidase digestion, peaks 9 and 11 from strawberry (corresponding to peaks 14 and 17 in celery, see Figure 3) were analyzed by 1D-^{1}H-NMR as pyridylaminated N-glycans.

For peak 11, putative $(GF)(GF)XF^3$, 1D-^{1}H-NMR spectroscopy showed the H-1, H-5, and CH_3 signals for three α-L-Fuc residues, the H-1 signals for two β-D-Gal residues, and the H-1 signals for two antennary β-D-GlcNAc residues (Table II). The values of the structural-reporter-group signals matched those reported by Takahashi *et al.* for laccase (Takahashi *et al.*, 1986) and miraculin (Takahashi *et al.*, 1990), and by Fitchette-Lainé *et al.* for laccase (Fitchette-Lainé *et al.*, 1997). The originally reported terminal sequence in laccase diantennary Xyl-containing N-glycans with α1,3-linked Fuc at the Asn-bound GlcNAc residue, Galβ–4(Fucα–6)GlcNAc (Takahashi *et al.*, 1986, 1990) was revised to be the Lewis^a epitope Galβ–3(Fucα–4)GlcNAc (Fitchette-Lainé *et al.*, 1997). Unfortunately, however, in a recent paper on peanut peroxidase glycans Shaw *et al.* followed the original interpretations of Takahashi *et al.* (Shaw *et al.*, 2000).

One of the fucose residues (H-1, δ 5.052; H-5, δ 4.231; and CH_3, δ 1.200) is α1,3-linked to the GlcNAc residue at the reducing end. The other two fucose residues with identical chemical shifts for the structural reporters (H-1, δ 5.007; H-5, δ 4.866; and CH_3, δ 1.176) are most comparable to α1,4-linked Fuc as present in O-glycan Le^a-determinants (H-1, δ 5.02–5.03; H-5, δ 4.86–4.88; and CH_3, δ 1.18) (Kamerling and Vliegenthart, 1992). The two Gal H-1 signals at δ 4.498 and δ 4.506 are in accordance with a Galβ1–3GlcNAc, rather than with a Galβ–4GlcNAc fragment. This conclusion fits with the insensitivity to β1,4-specific *Aspergillus* β-galactosidase of celery peak 17 after defucosylation with almond α-fucosidase.

Additionally, two 2D-^{1}H-NMR experiments were carried out, a total correlation spectroscopy (TOCSY) measurement for the assignment of the relevant skeleton protons and a nuclear Overhauser effect spectroscopy (NOESY) measurement for sequence/glycosidic linkage information. These data confirmed the presence of a β1,3-linkage between Gal and GlcNAc in both antennae. The chemical shifts of H-2, H-3, and H-4 of the α1,4-linked Fuc residues match those of an O-glycan Le^a-determinant (Strecker *et al.*, 1992). The H-1 signal of the antennary Fuc residues showed NOEs with the H-4, H-6a, and H-6b signals of the antennary GlcNAc residues, as shown to be the case for the synthetic Le^a-trisaccharide (Kogelberg and Rutherford, 1994). These findings thus constitute an illustration that caution should be applied when using NOEs as the sole basis for a structural assignment.

For strawberry peak 9, putative $(GF)GnXF^3/Gn(GF)XF^3$, the 1D-^{1}H-NMR also showed the presence of a Le^a-determinant (Fuc H-1, δ 5.008; Fuc H-5, δ 4.870; Fuc CH_3, δ 1.177). In view of the different structural-reporter-group signals for the antennary Gal and GlcNAc residues (Table II), this peak was determined to be a mixture of two isomers, with either a Le^a-determinant on the 6-branch and terminal GlcNAc on the 3-branch or vice versa (estimated ratio 3:1).

Oligomannosidic oligosaccharides

In initial studies, endoglycosidase H treatment of complete N-glycan pools from soya and pea extracts suggested that these extracts had a number of oligomannosidic oligosaccharides. Percentages of endoglycosidase H–sensitive glycans, as judged by the integration of the GlcNAc peak of 3.5 g.u. on reverse-phase were 78% and 63% for the extracts from soya and pea, respectively, whereas for other foods the percentage of oligomannose structures was far lower. Verification of the nature of the presumed oligomannosidic oligosaccharides was by means of (1) size estimation on Micropak; (2) comparison with experimentally determined retention times of the oligomannosidic series of soybean 7S glycoprotein (Man6–Man8), and Man5 from *A. oryzae* α-amylase; (3) demonstration that α-mannosidase treatment resulted in a final digestion product with retention time of 6.8 glucose units; and (4) by MALDI-TOF-MS.

In the case of three large pea peaks, the determined $[M+Na]^+$ masses were 1500.9, 1661.9, and 1824.4, consistent with the respective HPLC-determined designations of Man6 ($Man_6GlcNAc_2$), Man7, and Man8.

The oligomannosidic glycans from apple, celery, pea, soya, and strawberry were analyzed by 2D HPLC. For Man5 to Man8, a number of isomers can theoretically exist, although in the present study only multiple isomers of Man7 were found. For strawberry all three isomers of this structure were detected (5.2, 5.6, and 7.6 g.u.-RP). Based on the compatibility of the reverse-phase retention times with those given by Kubelka *et al.* (1994) and Tomiya *et al.* (1988), which fortunately agree with each other to within ± 0.1 g.u., the isomers were, in the order of their elution, assigned to be Man7(1), Man7(3), and Man7(2) (Altmann *et al.*, 1999) occuring in a ratio of 0.8 to 1.0 to 0.1 in strawberry.

Discussion

This large survey demonstrates that N-linked oligosaccharides known to be immunogenic are widespread in all vegetable food extracts tested. Though the clinical repercussions of this finding may be controversial, the presence of xylose and core α1,3-linked fucose on N-glycans in all these samples, as well

Table II. ^{1}H-NMR data of the constituent monosaccharides of N-glycans as present in strawberry fractions peak 11 [(GF)(GF)XF^3] and peak 9 [(GF)GnXF^3/Gn(GF)XF^3]. Chemical shifts are given at 300 K and were measured in ^2H_2O relative to internal acetone (δ 2.225). Residues are numbered as shown in Figure 1

Proton	Residue	(GF)(GF)XF^3	(GF)GnXF^3 / Gn(GF)XF^3	
H-1	GlcNAc-2	4.598	4.600	4.600
	Man-3	4.859	4.859	4.859
	Xyl	4.430	4.432	4.432
	Man-4	5.144	5.146	5.146
	Man-4′	4.907	4.905	4.905
	GlcNAc-5	4.540	4.540	4.552
	GlcNAc-5′	4.583	4.520	4.583
	Gal-6	4.498	4.499	—
	Gal-6′	4.506	—	4.507
	Fuc(α1-3)	5.052	5.052	5.052
	Fuc(α1-4)	5.007[a]	5.008	5.008
H-2	GlcNAc-1	4.468	4.472	4.472
	Man-3	4.247	4.249	4.249
	Man-4	4.150	4.151	4.151
	Man-4′	4.104	4.105	4.105
	GlcNAc-5	3.90[b]	ND[c]	ND
	GlcNAc-5′	3.90	ND	ND
	Gal-6	3.48	ND	—
	Gal-6′	3.48	—	ND
	Fuc(α1-4)	3.80[a]	ND	ND
H-3	GlcNAc-5	4.063	4.064	ND
	GlcNAc-5′	4.063	ND	4.064
	Gal-6	3.62	ND	—
	Gal-6′	3.62	—	ND
	Fuc(α1-4)	3.88[a]	ND	ND
H-4	GlcNAc-5	3.75	ND	ND
	GlcNAc-5′	3.75	ND	ND
	Gal-6	3.88	ND	—
	Gal-6′	3.88	—	ND
	Fuc(α1-4)	3.79[a]	ND	ND
H-5	GlcNAc-5	3.53	ND	ND
	GlcNAc-5′	3.53	ND	ND
	Fuc(α1-3)	4.231	4.234	4.234
	Fuc(α1-4)	4.866[a]	4.870	4.870
H-6a	GlcNAc-5	3.86	ND	ND
	GlcNAc-5′	3.86	ND	ND
H-6b	GlcNAc-5	3.97	ND	ND
	GlcNAc-5′	3.97	ND	ND
NAc	GlcNAc-1	1.946	1.944	1.944
	GlcNAc-2	2.048	2.050	2.050
	GlcNAc-5	2.048	2.050	2.050
	GlcNAc-5′	2.048	2.050	2.050
CH_3	Fuc(α1-3)	1.200	1.200	1.200
	Fuc(α1-4)	1.176[a]	1.177	1.177

[a]Signal stemming from two residues.
[b]In case of two decimals, the data are derived from a 2D TOCSY experiment.
[c]ND, not determined.

as their previously shown presence on N-glycans of pollens (Wilson and Altmann, 1998), indicate that asparagine-linked oligosaccharides are a probable major source of carbohydrate-mediated cross-reactions between foods, as well as between foods and pollens.

Utility of glycome analysis of foods

The results presented in this article were primarily obtained by a novel method for preparation and purification of N-glycans from whole foodstuffs. This strategy is believed to avoid two problems inherent to the currently applied procedures of glycoprotein analysis: (1) enzymatic degradation of N-glycans during extraction and purification of glycoproteins and (2) preferential detection of N-glycan species present on abundant, soluble glycoproteins. The data presented here for a wide variety of edible parts of food crops derived from various dicotyledons and monocotyledons as well as one gymnospermic plant therefore may well reflect the general glycosylation capacity of this tissue at a given time. By analogy to its older "cousins" genome and proteome analysis we thus would like to call the herein presented strategy *glycome analysis*, in which the glycome is represented by a mass profile obtained by MALDI-TOF-MS. Knowledge of the structures and the biosynthesis of N-glycans in plants is utilized to translate mass data into compositions and structure (Lerouge *et al.*, 1998). We have tested the suitability of these tentative assignments by separating and analyzing the glycans from a number of samples by additional methods, such as HPLC of pyridylaminated glycans and ^{1}H-NMR. Generally, the quantitative and qualitative results obtained by MALDI-TOF-MS and by HPLC agreed, notwithstanding the ability of RP-HPLC to resolve isomeric structures such as MGnXF^3/GnMXF^3 or M(GF)XF^3/(GF)MXF^3. Only in the case of trace amounts of (GF)FXF^3 and FFXF^3 from celery, which were detected by MALDI-TOF-MS in fractions purified by HPLC but not in the whole mixture, did the results deviate. As the masses of these compounds ($[M+Na]^+ = 2072.7$ and 1901.7) are close to that of more abundant glycans, detection of these trace compounds was beyond the limits of MALDI-TOF-MS with a linear flight path. As the presented method for glycome analysis is very new, the results cannot be directly compared with published data. Only in the case of tomato, a recent publication in general validates the structural assignments from the glycan masses. However, it also reveals the discrepancies between results obtained with different methods of N-glycan preparation (see *Results*). Another similar study has been performed on soluble extracts from allergenic pollens (Wilson and Altmann, 1998). In this case, no structures larger GnGnXF^3 were found, and thus it is possible to ask whether the complete lack of such larger structures was likewise an artifact of allergen extraction rather than a reflection of the pollens' authentic N-glycan profile.

A remarkable situation is found with soybeans, where the N-glycans obtained from the entire beans qualitatively and quantitatively essentially resemble to those on the 7S major storage protein (Neeser *et al.*, 1985). In some samples, oligomannosidic N-glycans carrying 10 hexose residues were found. Considering the biosynthesis of N-glycans and a recent paper on Glc-containing Man9 in jack bean α-mannosidase (Kimura *et al.*, 1999), the 10th hexose is presumed to be glucose. For papaya leaves, the occurence of the unusual structures Man5XF^3 and Man5GnXF^3 has been reported (Makino *et al.*, 2000). These N-glycans have also been found as major constituents of papaya fruit (Table I). In the present study, further elaborations of the 3-arm leading to the novel structures Man5GXF^3 and Man5(GF)XF^3 could be shown for a small fraction of papaya fruit N-glycans.

A severe limitation of MALDI-TOF-MS is that isobaric structures and monosaccharides, such as arabinose and xylose, cannot be distinguished. One study suggested the presence of arabinose in free tomato N-glycans (Priem *et al.*, 1993). HPLC fractionation did not yield fractions with an elution behavior deviating from the Xyl-containing standards. However, Ara-containing glycans might comigrate; but compositional analysis of MMXF^3 and GnGnXF^3 from celery revealed the presence of xylose only. Xylose-containing glycans that carry additional rhamnose and/or arabinose residues have been suggested to be present on secreted carrot glycoproteins (Sturm, 1991); in the present study, however, masses indicative of such structures were not found during the analysis of carrot N-glycans.

Glycome analysis is, of course, only a first step in the determination of which glycans have a role in allergy and IgE cross-reactivity. One limitation of most studies in this area, including the present one, is that O-glycans have been generally ignored, although there is data suggesting a role for O-glycans as IgE epitopes on the major allergen of *Parthenium* pollen (Gupta *et al.*, 1996). Unfortunately, methods developed for the analysis of mammalian O-glycans are inapplicable to plant O-glycans due to the entirely different linkages of sugar to protein (Klis, 1995). Studies are being initiated to investigate these glycans in a range of foods, which may prove to be another source of cross-reactivity.

Presence of Le^a

The literature on plant glycosylation has until recently given the impression that plant N-linked oligosaccharides are either of the truncated "vacuolar" type, with core α1,3-linked fucose and/or β1,2-linked xylose, or are oligomannosidic. Until 1997, only three instances of larger structures had been reported. These glycans, suggested to carry the nonreducing terminal Galβ–4(Fucα–6)GlcNAc sequence were found in highly "exotic" sources, that is, sycamore cell laccase, the taste-modifying protein miraculin, and Japanese cedar pollen allergen Cry j I (Takahashi *et al.*, 1986, 1990; Ogawa *et al.*, 1996). However, more recently it has become apparent that these terminal fucosylated sequences take the form Galβ–3(Fucα–4)GlcNAc, the same as and cross-reacting with antibodies against mammalian Le^a structures (Fitchette-Lainé *et al.*, 1997; Melo *et al.*, 1997). The combined HPLC and NMR data from the present study are consistent with the revisionist interpretation of the data on plant glycans with branches containing galactose and fucose residues.

Antibody-binding studies have shown that the Le^a epitope is present in a range of plant tissues, such as onion and tomato roots, sycamore, gingko, spruce, crocus, walnut, chive, celery, and columbine leaves, while being completely or nearly absent from *Arabidopsis*, canola, radish, and cauliflower (Fitchette-Lainé *et al.*, 1997; Fitchette *et al.*, 1999). In keeping with these results, no Le^a-containing glycans could be found in cauliflower by MALDI-TOF-MS (Table I). However, papaya (which, like *Arabidopsis* and cauliflower, belongs to the Brassicaceae family) did exhibit Le^a determinants albeit as part of a unique

type of hybrid N-glycans (Table I, Figure 1). Mass spectrometry data also indicate the presence of the Le^a epitope in tobacco (Fitchette *et al.*, 1999), an important plant with respect to expression of recombinant proteins. As shown for the first time by direct structural analysis, the N-glycan repertoire of gymnospermic plants (e.g., conifers) resembles that from angiospermic plants, including the presence of smaller xylosylated and core-α1,3-fucosylated as well as of larger Le^a-containing N-glycans. In contrast to structural analyses by others (Kimura and Matsuo, 2000) but in agreement with the aforementioned antibody-binding data (Fitchette-Lainé *et al.*, 1997), our recent data (Kolarich, D., unpublished observations) indicate the presence of the Le^a structure in gingko, another old phylogenetic neighbor of the angiospermic plants.

Our results constitute direct structural confirmation that the Le^a epitope is widespread and that members of a wide spectrum of taxonomic orders are capable of synthesizing this determinant. Le^a-carrying glycans were major components primarily in "fleshy" foods, that is, in apple, asparagus, banana, carrot, celery, kiwi, onion, orange, pear, and strawberry. Morever, pignoli (from a coniferous plant), avocado, hazelnut, and walnut contained appreciable amounts of these glycans, whereas tomato and potato (Solanaceae) and legume seeds generally contained only small amounts of Le^a-carrying glycans. Furthermore, our data indicating the presence of the Le^a-epitope in kiwi is compatible with the interaction of glycoproteins from this fruit with *Aleuria aurantia* lectin (Fahlbusch *et al.*, 1998), which binds a number of fucose-containing oligosaccharides including some carrying Le^a (Kochibe and Furukawa, 1982). The presence of this epitope raises questions as to its effect on animals exposed to plant material expressing these structures.

Comparison with antibody data

The presence of xylose and core α1,3-linked fucose in all tested foods is consistent with our previous findings (Wilson *et al.*, 1998) on the binding of anticarbohydrate antibodies to a range of soluble vegetable, fruit, and nut extracts. In the present study, direct comparison can be made between the antibody data (using anti–horseradish peroxidase and the core α1,3-linked fucose-specific monoclonal YZ1/2.23) and the structural data for pea, soya, avocado, pear, strawberry, almond, coconut, and pistachio. All these extracts showed high binding to anti–horseradish peroxidase, in general inhibitable by the addition of a conjugate of bovine serum albumin with a glycopeptide carrying $MUXF^3$ but not inhibitable by the defucosylated analogue conjugate. In addition all of these samples, except pea, showed significant binding (also inhibitable) to YZ1/2.23. Although pea and coconut both carry very little $MMXF^3$, there is a comparably high binding of YZ1/2.23 to coconut where glycans with xylose only predominate. Thus, although nonfucosylated glycopeptide-albumin conjugates were poor inhibitors for the binding of YZ1/2.23 to $MUXF^3$ structures (Wilson *et al.*, 1998), solely xylosylated N-glycans like MMX nevertheless might have affinity to this monoclonal. Another explanation would be that $MMXF^3$ determinants are presented more properly by the extract from coconut than that from pea. In the literature there are other reports that polyclonal antibodies against carrot fructosidase (Faye and Chrispeels, 1988) and peanut peroxidase (Wan and van Huystee, 1994) are also predominantly "anticarbohydrate"; the former antibody's binding to apple and carrot is consistent with the present data.

Knowledge of the glycan structures, rather than just antibody-binding data, present in the complete mix means that further research can be better directed. Approaches with promise include direct analysis of glycans on allergens separated by SDS–polyacrylamide gel electrophoresis (Kolarich and Altmann, 2000), as well as the use of specific glycoconjugates as inhibitors of IgE binding or RAST assays or as elicitors of histamine release.

Materials and methods

Materials

If not otherwise stated, the fruits, vegetables or seeds were purchased at local supermarkets: sweet almonds (*Prunus dulcis* var. *sativa*), apple (*Malus domestica*, var. Golden Delicious), asparagus (*Asparagus officinalis*), avocado (*Persea americana*), banana (*Musa acuminata*), buckwheat (*Fagopyrum esculentum*), carrot (*Daucus carota*), cauliflower (*Brassica oleracea* var. *botrytis*), celery tuber (*Apium graveolens*), coconut (*Cocos nucifera*), common mushroom (*Agaricus bisporus*), hazelnut (*Corylus avellana*), kiwi (*Actinidia chinensis*), mung bean (*Vigna radiata*; dry beans), onion (*Allium cepa*), orange (*Citrus sinensis*; peeled), papaya (*Carica papaya*), pea (*Pisum sativa*; frozen green peas), peanut (*Arachis hypogaea*), pear (*Pyrus communis*, var. Williams), pine nut (*Pinus pinea*), pistachio (*Pistacia vera*), potato (*Solanum tuberosum*; new harvest, peeled), soya (*Glycine max*), strawberry (*Fragaria x ananassa*), tomato (*Lycopersicon esculentum*), and walnuts (*Juglans regia*). Lyophilized soluble extracts of avocado, almond, coconut, pea, pistachio, and soya, which were also used in a previous study (Wilson *et al.*, 1998), were the kind gift of Professor Christof Ebner (Allgemeines Krankenhaus der Stadt Wien). Pyridylaminated oligosaccharides of known structure from previous studies and derived from horseradish peroxidase ($MMXF^3$), bromelain ($MUXF^3$), and ascorbate oxidase fraction 1 (MMX) were used as standards (for an explanation of oligosaccharide nomenclature see Figure 1). A mixture of N-glycans from sycamore cell laccase was kindly provided by Dr. Patrice Lerouge (Mt. St. Aignan, France). Almond peptide:N-glycosidase A was purified as described (Altmann *et al.*, 1998) or obtained from Boehringer Mannheim. Other materials were purchased from Merck or Sigma-Aldrich.

Preparation of N-glycans

Approximately 100 g of fleshy fruit or vegetable (e.g., apple or carrot) or 40 g of dry foods (e.g., nuts and beans) were chopped or ground and suspended in 200 ml of water. After mixing with a kitchen blender, the slurries were made up to 300 ml with a final concentration of 5% (v/v) formic acid and about 0.1 mg/ml pepsin (and 0.1% 2-mercaptoethanol in the case of potato). After prewarming in a water bath, the slurry was incubated at 37°C for 20 h with occasional stirring. Insoluble material was then removed by centrifugation (20 min, 15°C, 10,000 rpm, Sorvall-DuPont GSA rotor). The supernatant was mixed for 15 min with 100 ml Dowex 50W ×2 (prewashed with 500 ml 0.4 M ammonium acetate, pH 6.0, and 1 L 2% [v/v] acetic acid). The resin was then washed in a funnel with 300 ml 2%

(v/v) acetic acid, poured into a column, washed with one column volume of 2% (v/v) acetic acid and then with 0.4 M ammonium acetate, pH 6.0. Forty fractions of 400 drops were collected, and fractions that gave a positive orcinol test were pooled and the volume reduced by rotary evaporation. This glycopeptide fraction was subject to gel filtration on Sephadex G25 (coarse; 2.5 × 120 cm) and eluted with 1% (v/v) acetic acid. The orcinol-positive fractions were pooled, lyophilized, and dissolved in approximately 1 ml citrate-phosphate buffer, pH 5.0. After incubation at 95°C for 10 min, the N-glycans were released by incubation with 0.7 U of peptide:N-glycosidase A, 37°C, 24 h. An equal volume of 10% (v/v) acetic acid was added to acidify the sample, which was then passed over Dowex 50W (4–5 ml) to separate released N-glycans from residual glycopeptides (including possible O-linked glycans). The column was washed with 2% (v/v) acetic acid; the unretained orcinol positive fractions were concentrated and subject to gel filtration (Sephadex G15, elution with 1% [v/v] acetic acid). The orcinol positive fractions were pooled, concentrated, and applied to a small reversed-phase column (200 µl; Lichroprep RP 18, 25–40 µm prewashed with 2 ml of 60% [v/v] propan-2-ol in water containing 5% [v/v] acetic acid) and then 5 ml 5% [v/v] acetic acid). The N-glycans were eluted with 2–3 ml 5% (v/v) acetic acid, concentrated with a rotary evaporator, lyophilized, and finally dissolved in 50 µl water for MALDI-TOF-MS analysis.

In some cases, the glycans were then subject to derivatization by reductive pyridylamination (Hase *et al.*, 1984; Kubelka *et al.*, 1993; Wilson and Altmann, 1998). N-glycans from extracted soluble glycoproteins were prepared as described for pollen extracts (Wilson and Altmann, 1998).

MALDI-TOF-MS

Aliquots of 0.8 µl of underivatized or pyridylaminated N-glycans were applied to a flat sample platen. To the dry sample, 0.8 µl of matrix (2% 2,5-dihydroxybenzoic acid in 30% [v/v] acetontrile) were added and and dried immediately under mild vacuum. MALDI-TOF-MS spectra were acquired on a DYNAMO (Thermo BioAnalysis, Santa Fe, NM) linear time-of-flight mass spectrometer capable of dynamic extraction, a synonym for delayed extraction. The instrument was operated with a dynamic extraction setting of 0.1. External mass calibration was performed with pyridylaminated N-glycans or with a partial dextran hydrolysate.

HPLC analysis and glycosidase digestions

Pyridylaminated oligosaccharides were fractionated by a "two-dimensional" mapping technique starting with separation according to size on a Micropak AX-5 column (0.4 × 30 cm) (Tomiya *et al.*, 1988). Peaks were collected and dried thoroughly prior to subfractionation in the second dimension by reverse-phase chromatography on an Hypersil ODS column (0.4 × 25 cm) (Kubelka *et al.*, 1993; Wilson and Altmann, 1998). Columns were calibrated daily in terms of glucose units with a pyridylaminated partial dextran hydrolysate (3–10 glucose units). Peaks from either size fractionation or reverse-phase chromatographies were subject to exo- or endoglycosidase digestions as follows: *Canavalia ensiformis* (jack bean) α-mannosidase ("medium" dose; 25 mU in 20 µl 50 mM sodium acetate, 0.1 mM zinc chloride, pH 4.2); *C. ensiformis* β-hexosaminidase (25 mU in 20 µl 0.1 M sodium citrate, pH 5.0); endoglycosidase H (2 mU in 20 µl, 0.1 M citrate-phosphate, pH 5.0); almond α-fucosidase 1 µl (0.1 µU in 20 µl, 0.1 M citrate-phosphate, pH 5.0); bovine testes β-galactosidase 2 µl (1.6 mU in 20 µl, 0.1 M citrate-phosphate, pH 5.0).

Monosaccharide analysis

Sugars were analyzed as alditol acetates by GLC-MS as described (Kubelka *et al.*, 1993).

^{1}H-NMR spectroscopy

The oligosaccharide samples were exchanged twice with ^2H_2O (99.9 atom% ^{2}H, Cambridge Isotope Ltd.) with intermediate lyophilisation, then dissolved in ^2H_2O (99.96 atom% ^{2}H, Isotec Inc.). 1D-^{1}H-NMR spectra were recorded at 600 MHz on a Bruker AMX 600 instrument (Bijvoet Center, Department of NMR Spectroscopy, Utrecht University) at probe temperatures of 300 K. Chemical shifts (δ) are expressed in p.p.m. by reference to internal acetone (δ 2.225). A 2D TOCSY spectrum at 600 MHz was recorded using Bruker software with a MLEV-17 spin-lock pulse sequence (100 ms) preceded by a 2.5-ms trim pulse, and a 2D NOESY spectrum was recorded using a mixing time of 400 ms. Data matrices of 512 × 1024 points were collected representing a spectral width of 4800 Hz in each dimension. The residual water signal was suppressed by presaturation for 1 s during the relaxation delay. Phase-sensitive handling of the data was performed by the TPPI method inplemented in the Bruker software. The time domain data were zero-filled to data matrices of 1024 × 1024 points, prior to multiplication with a phase-shifted square bell function.

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Abbreviations

ELISA, enzyme-linked immunoassay; GLC-MS, gas liquid chromatography–mass spectrometry; g.u., glucose units; g.u.-MP, glucose units on Micropak sizing column; g.u.-RP; glucose units on reversed phase column; HPLC, high-performance liquid chromatography; MUX, (GF)GnXF^3 and similar terms, N-glycans as specified in Figure 1; MALDI-TOF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; NMR, nuclear magnetic resonance; NOESY, nuclear Overhauser effect spectroscopy; NOE, nuclear Overhauser effect; RAST, radioallergosorbent tests; RP-HPLC, reverse-phase high-performance liquid chromatography; TOCSY, total correlation spectroscopy.

References

Aalberse, R.C. (1997) Food allergens. *Env. Toxicol. Pharmacol.*, **4**, 55–60.

Aalberse, R.C., and van Ree, R. (1997) Crossreactive carbohydrate determinants. *Clin. Rev. Allergy Immunol.*, **15**, 375–387.

Ahlroth, M., Alenius, H., Turjanmaa, K., Mäkinen-Kiljunen, S., Reunala, T., and Palosuo, T. (1995) Cross-reacting allergens in natural rubber latex and avocado. *J. Allergy Clin. Immunol.*, **96**, 167–173.

Altmann, F., Paschinger, K., Dalik, T., and Vorauer, K. (1998) Characterisation of peptide-N^4-(*N*-acetyl-β- glucosaminyl)asparagine amidase A and its N-glycans. *Eur. J. Biochem.*, **252**, 118–123.

Altmann, F., Staudacher, E., Wilson, I.B.H., and März, L. (1999) Insect cells as hosts for the expression of recombinant glycoproteins. *Glycoconjugate J.*, **16**, 109–123.

Batanero, E., Crespo, J.F., Monsalve, R.I., Martin-Esteban, M., Villalba, M., and Rodríguez, R. (1999) IgE-binding and histamine-release capabilities of the main carbohydrate component isolated from the major allergen of olive tree pollen, Ole e 1. *J. Allergy Clin. Immunol.*, **103**, 147–153.

Bernhisel-Broadbent, J. (1995) Allergenic cross-reactivity of foods and characterisation of food allergens and extracts. *Ann. Allergy Asthma Immunol.*, **75**, 295–303.

Blanco, C., Carrillo, T., Castillo, R., Quiralte, J., and Cuevas, M. (1994) Avocado hypersensitivity. *Allergy*, **49**, 454–459.

Breiteneder, H. (1998) Plant-food and seafood allergens—an overview. *Allergy*, **53**, 31–34.

Burks, W., Sampson, H.A., and Bannon, G.A. (1998) Peanut allergens. *Allergy*, **53**, 725–730.

Calkhoven, P.G., Aalbers, M., Koshte, V., Pos, O., Oei, H., and Aalberse, R. (1987) Cross-reactivity among birch pollen, vegetables and fruits as detected by IgE antibodies is due to at least three distinct cross-reactive structures. *Allergy*, **42**, 382–390.

de las Marinas, D., Vila, L., and Sanz, M.L. (1998) Allergy to pine nuts. *Allergy*, **53**, 220–222.

de Maat-Bleeker, D., and Stapel, S.O. (1998) Cross-reactivity between buckwheat and latex. *Allergy*, **53**, 538–539.

Diaz-Perales, A., Collada, C., Blanco, C., Sanchez-Monge, R., Carrillo, T., Aragoncillo, C., and Salcedo, G. (1999) Cross-reactions in the latex-fruit syndrome: a relevant role of chitinases but not of complex asparagine-linked glycans. *J. Allergy Clin. Immunol.*, **104**, 681–687.

Ebner, C., Hirschwehr, R., Bauer, L., Breiteneder, H., Valenta, R., Hoffmann, K., Krebitz, M., Kraft, D. and Scheiner, O. (1996) Identification of allergens in apple, pear, celery, carrot and potato. In: Wüthrich, B., and Ortolani, C. (eds.), *Highlights in food allergy*. Karger, Basel, Vol. 32, pp. 73–77.

Eng, P.A., Yman, L., Maaninen, E., and Wüthrich, B. (1996) Inhalant allergy to fresh asparagus. *Clin. Exp. Allergy*, **26**, 330–334.

Escribano, M.M., Munoz-Bellido, F.J., Serrano, P., A., de la Calle, A., and Conde, J. (1998) Acute urticaria after ingestion of asparagus. *Allergy*, **53**, 622–623.

Fahlbusch, B., Rudeschko, O., Schumann, C., Steurich, F., Henzgen, M., Schlenvoigt, G., and Jäger, L. (1998) Further characterisation of IgE-binding antigens in kiwi, with particular emphasis on glycoprotein allergens. *J. Investig. Allergol. Clin. Immunol.*, **8**, 325–332.

Faye, L., and Chrispeels, M.J. (1988) Common antigenic determinants in the glycoproteins of plants, molluscs and insects. *Glycoconj. J.*, **5**, 245–256.

Fernandez, C., Fiandor, A., Martinez-Garate, A., and Quesada, J.M. (1995) Allergy to pistachio: cross-reactivity between pistachio nut and other Anacardiaceae. *Clin. Exp. Allergy*, **25**, 1254–1259.

Fernández-Rivas, M., van Ree, R., and Cuevas, M. (1997) Allergy to Rosaceae fruits without related pollinosis. *J. Allergy Clin. Immunol.*, **100**, 728–733.

Fitchette, A.C., Cabanes-Macheteau, M., Marvin, L., Martin, B., Satiat-Jeune-maitre, B., Gomord, V., Crooks, K., Lerouge, P., Faye, L., and Hawes, C. (1999) Biosynthesis and immunolocalisation of Lewis a-containing *N*-glycans in the plant cell. *Plant Physiol.*, **121**, 333–343.

Fitchette-Lainé, A.C., Gomord, V., Cabanes, M., Michalski, J.C., Saint Macary, M., Foucher, B., Cavelier, B., Hawes, C., Lerouge, P., and Faye, L. (1997) N-glycans harboring the Lewis a epitope are expressed at the surface of plant cells. *Plant J.*, **12**, 1411–1417.

Fötisch, K., Altmann, F., Haustein, D., and Vieths, S. (1999) Involvement of carbohydrate epitopes in the IgE response of celery-allergic patients. *Int. Arch. Allergy Immunol.*, **120**, 30–42.

Fuchs, T., Spitzauer, S., Vente, C., Hevler, J., Kapiotis, S., Rumpold, H., Kraft, D., and Valenta, R. (1997) Natural latex, grass pollen, and weed pollen share IgE epitopes. *J. Allergy Clin. Immunol.*, **100**, 356–364.

Gall, H., Kalveram, K., Forck, G., and Sterry, W. (1994) Kiwi fruit allergy: a new birch pollen-associated food allergy. *J. Allergy Clin. Immunol.*, **94**, 70–76.

Grattan, C.E.H., and Harman, R.R.M. (1985) Contact urticaria to strawberry. *Contact Dermatitis*, **13**, 191–192.

Gupta, N., Martin, B.M., Metcalfe, D.D., and Rao, P.V.S. (1996) Identification of a novel hydroxyproline-rich glycoprotein as the major allergen in *Parthenium* pollen. *J. Allergy Clin. Immunol.*, **98**, 903–912.

Hase, S., Ibuki, T., and Ikenaka, T. (1984) Reexamination of the pyridyl-amination used for fluorescence labelling of oligosaccharides and its application to glycoproteins. *J. Biochem. (Tokyo)*, **95**, 197–203.

Heiss, S., Fischer, S., Müller, W.-D., Weber, B., Hirschwehr, R., Spitzauer, S., Kraft, D., and Valenta, R. (1996) Identification of a 60 kd cross-reactive allergen in pollen and plant-derived food. *J. Allergy Clin. Immunol.*, **98**, 938–947.

Helbling, A. (1997) Wichtige kreuzreaktive Allergene. *Schweiz Med. Wochenschrift*, **127**, 382–389.

Hirschwehr, R., Valenta, R., Ebner, C., Ferreira, F., Sperr, W.R., Valent, P., Rohac, M., Rumpold, H., Scheiner, O., and Kraft, D. (1992) Identification of common allergenic structures in hazel pollen and hazelnuts: A possible explanation for sensitivity to hazelnuts in patients allergic to tree pollen. *J. Allergy Clin. Immunol.*, **90**, 927–936.

Jankiewicz, A., Aulepp, H., Altmann, F., Fötisch, K., and Vieths, S. (1998) Serological investigation of 30 celery-allergic patients with particular consideration of the thermal stability of IgE-binding celery allergens. *Allergo J.*, **7**, 87–95.

Kamerling, J.P., and Vliegenthart, J.F.G. (1992). High-resolution H-1-nuclear magnetic resonance spectroscopy of oligosaccharide-alditols released from mucin-type O-glycoproteins. *Biol. Magn. Reson.*, **10**, 1–194.

Kimura, Y., and Matsuo, S. (2000) Changes in N-linked oligosaccharides during seed development of *Ginkgo biloba*. *Biosci. Biotechnol. Biochem.*, **64**, 562–568.

Kimura, Y., Hess, D., and Sturm, A. (1999) The N-glycans of jack bean α-mannosidase. *Structure*, topology and function. *Eur. J. Biochem.*, **264**, 168–175.

Klis, F.M. (1995) O-glycosylation in plants. In: Montreuil, J., Schachter, H., and Vliegenthart, J.F.G. (eds.), *Glycoproteins*. Elsevier Science, pp. 511–520.

Kochibe, N., and Furukawa, K. (1982) *Aleuria aurantia* haemagglutinin. *Methods Enzymol.*, **83**, 373–377.

Kogelberg, H., and Rutherford, T.J. (1994) Studies on the three-dimensional behaviour of the selectin ligands Lewis a and sulphated Lewis a using NMR spectroscopy and molecular dynamics simulations. *Glycobiology*, **4**, 49–57.

Kolarich, D., and Altmann, F. (2000) N-glycan analysis by matrix assisted laser desorption/ionisation mass spectrometry of electrophoretically separated non-mammalian proteins. Application to peanut allergen Ara h 1 and olive pollen allergen Ole e 1. *Anal. Biochem.*, **285**, 64–75.

Kubelka, V., Altmann, F., Kornfeld, G., and März, L. (1994) Structures of the N-linked oligosaccharides of the membrane glycoproteins from three lepidopteran cell lines (Sf-21, IZD-Mb-0503, Bm-N). *Arch. Biochem. Biophys.*, **308**, 148–157.

Kubelka, V., Altmann, F., Staudacher, E., Tretter, V., März, L., Hård, K., Kamerling, J.P., and Vliegenthart, J.F. (1993) Primary structures of the N-linked carbohydrate chains from honeybee venom phospholipase A_2. *Eur. J. Biochem.*, **213**, 1193–1204.

Lavaud, F., Prevost, A., Cossart, C., Guerin, L., Bernard, J., and Kochman, S. (1995) Allergy to latex, avocado pear, and banana: evidence for a 30 kd antigen in immunoblotting. *J. Allergy Clin. Immunol.*, **95**, 557–564.

Lerouge, P., Cabanes-Macheteau, M., Rayon, C., Fitchette-Lainé, A.C., Gomord, V., and Faye, L. (1998) N-glycoprotein biosynthesis in plants: recent developments and future trends. *Plant Mol. Biol.*, **38**, 31–48.

Liccardi, G., Mistrello, G., Noschese, P., Falagiani, P., D'Amato, M., and D'Amato, G. (1996) Oral allergy syndrome (OAS) in pollinosis patients after eating pistachio nuts. *Allergy*, **51**, 919–992.

Makino, Y., Shimazaki, A., Omichi, K., Odani, S. and Hase, S. (2000) Processing pathway deduced from the structures of N-glycans in *Carica papaya*. *J. Biochem. (Tokyo)*, **127**, 1121–1126.

Mari, A., Iacovacci, P., Afferni, C., Barletta, B., Tinghino, R., di Felice, G., and Pini, C. (1999) Specific IgE to cross-reactive carbohydrate determinants strongly affect the *in vitro* diagnosis of allergic diseases. *J. Allergy Clin. Immunol.*, **103**, 1005–1011.

Melo, N.S., Nimtz, M., Conradt, H.S., Fevereiro, P.S., and Costa, J. (1997) Identification of the human Lewis^a carbohydrate motif in a secretory

peroxidase from a plant cell suspension culture (*Vaccinium myrtillus* L.). *FEBS Lett.*, **415**, 186–191.

Möller, M., Kayama, M., Vieluf, D., Paschke, A., and Steinhart, H. (1998) Determination and characterisation of cross-reacting allergens in latex, avocado, banana, and kiwi fruit. *Allergy*, **53**, 289–296.

M'Raihi, L., Charpin, D., Pons, A., Bongrand, P., and Vervloet, D. (1991) Cross-reactivity between latex and banana. *J. Allergy Clin. Immunol.*, **87**, 129–130.

Neeser, J.R., Vedovo, S., Mutsaers, J.H.G.M., and Vliegenthart, J.F.G. (1985) Structural analysis of the carbohydrate chains of legume storage proteins by 500-MHz ^{1}H-NMR spectroscopy. *Glycoconj. J.*, **2**, 355–364.

Ogawa, H., Hijikata, A., Amano, M., Kojima, K., Fukushima, H., Ishizuka, I., Kurihara, Y., and Matsumoto, I. (1996) Structures and contribution to the antigenicity of oligosaccharides of Japanese cedar (*Cryptomeria japonica*) pollen allergen *Cry j* I: Relationship between the structures and antigenic epitopes of plant *N*-linked complex-type glycans. *Glycoconj. J.*, **13**, 555–566.

Oku, H., Hase, S., and Ikenaka, T. (1990) Separation of oligomannose-type sugar chains having one to five mannose residues by high-performance liquid chromatography as their pyridylamino derivatives. *Anal. Biochem.*, **185**, 331–334.

Parra, F.M., Cuevas, M., Lezaun, A., Alonso, M.D., Beristain, A.M., and Losada, E. (1993) Pistachio nut hypersensitivity: identification of pistachio nut allergens. *Clin. Exp. Allergy*, **23**, 996–1001.

Petersen, A., Vieths, S., Aulepp, H., Schlaak, M., and Becker, W.M. (1997) Ubiquitous structures responsible for IgE cross-reactivity between tomato fruit and grass pollen allergens. *J. Allergy Clin. Immunol.*, **98**, 805–815.

Priem, B., Gitti, R., Bush, C.A., and Gross, K.C. (1993) Structure of ten free *N*-glycans in ripening tomato fruit. Arabinose is a constituent of a plant *N*-glycan. *Plant Physiol.*, **102**, 445–458.

Salcedo, G., Diaz-Perales, A., and Sanchez-Monge, R. (1999) Fruit allergy: plant defence proteins as novel potential panallergens. *Clin. Exp. Allergy*, **29**, 1158–1160.

Schöning, B., Vieths, S., Petersen, A., and Baltes, W. (1995) Identification and characterisation of allergens related to *Bet v* I, the major birch pollen allergen, in apple, cherry, celery and carrot by two-dimensional immunoblotting and *N*-terminal microsequencing. *J. Sci. Food. Agric.*, **67**, 431–440.

Shaw, G.S., Sun, Y., Barber, K.R., and van Huystee, R.B. (2000) Sequence specific analysis of the heterogeneous glycan chain from peanut peroxidase by ^{1}H-NMR spectroscopy. *Phytochemistry*, **53**, 135–144.

Sicherer, S.H., Burks, A., and Sampson, H. (1999) Clincal features of acute allergic reactions to peanut and tree nuts in children. *Pediatrics*, **102**, e6.

Strecker, G., Fievre, S., Wieruszeski, J.M., Michalski, J.C., and Montreuil, J. (1992) Primary structure of four human milk octa-, nona-, and undecasaccharides established by ^{1}H- and ^{13}C-nuclear magnetic resonance spectroscopy. *Carbohydr. Res.* **226**, 1–14.

Sturm, A. (1991) Heterogeneity of the complex N-linked oligosaccharides at specific glycosylation sites of two secreted carrot glycoproteins. *Eur. J. Biochem.*, **199**, 169–179.

Takahashi, N., Hitotsuya, H., Hanzawa, H., Arata, Y., and Kurihara, Y. (1990) Structural study of asparagine-linked oligosaccharide moiety of taste-modifying protein, miraculin. *J. Biol. Chem.*, **265**, 7793–7798.

Takahashi, N., Hotta, T., Ishihara, H., Mori, M., Tejima, S., Bligny, R., Akazawa, T., Endo, S., and Arata, Y. (1986) Xylose-containing common structural unit in N-linked oligosaccharides of laccase from sycamore cells. *Biochemistry*, **25**, 388–395.

Teuber, S.S., and Peterson, W.R. (1999) Systemic allergic reaction to coconut (*Cocos nucifera*) in 2 subjects with hypersensitivity to tree nut and demonstration of cross-reactivity to legumin-like seed storage proteins: new coconut and walnut allergens. *J. Allergy Clin. Immunol.*, **103**, 1180–1185.

Tomiya, N., Awaya, J., Kurono, M., Endo, S., Arata, Y., and Takahashi, N. (1988) Analyses of N-linked oligosaccharides using a two-dimensional mapping technique. *Anal. Biochem.*, **171**, 73–90.

Tomiya, N., Lee, Y.C., Yoshida, T., Wada, Y., Awaya, J., Kurono, M., and Takahashi, N. (1991) Calculated two-dimensional sugar map of pyridylaminated oligosaccharides: Elucidation of the jack bean α-mannosidase digestion pathway of $Man_9GlcNAc_2$. *Anal. Biochem.*, **193**, 90–100.

Valdivieso, R., Subiza, J., Varela-Losada, S., Subiza, J.L., Narganes, M.J., Martinez-Cocera, C., and Cabrera, M. (1994) Bronchial asthma, rhinoconjunctivitis, and contact dermatitis caused by onion. *J. Allergy Clin. Immunol.*, **94**, 928–930.

Valenta, R., Duchene, M., Ebner, C., Valent, C., Sillaber, C., Deviller, P., Ferreira, F., Tejkl, M., Edelmann, H., and Kraft, D. (1992) Profilins constitute a novel family of functional plant pan-allergens. *J. Exp. Med.*, **175**, 377–385.

Van der Veen, M.J., Van Ree, R., Aalberse, R.C., Akkerdaas, J., Koopelman, S.J., Jansen, H.M., and Van der Zee, J.S. (1997) Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. *J. Allergy Clin. Immunol.*, **100**, 327–334.

van Ketel, W.G. (1975) A cauliflower allergy. *Contact Dermatitis*, **1**, 324–325.

van Ree, R., and Aalberse, R.C. (1993) Pollen-vegetable food cross-reactivity: serological and clinical relevance of crossreactive IgE. *J. Clin. Immunoassay*, **16**, 124–130.

van Ree, R., and Aalberse, R.C. (1999) Specific IgE without clinical allergy. *J. Allergy Clin. Immunol.*, **103**, 1000–1001.

van Ree, R., Cabanes-Macheteau, M., Akkerdaas, J., Milazzo, J.P., Loutelier-Bourhis, C., Rayon, C., Villalba, M., Koppelman, S., Aalberse, R., Rodríguez, R., and others (2000) β(1, 2)-xylose and α(1, 3)-fucose residues have a strong contribution in IgE binding to plant glycoallergens. *J. Biol. Chem.*, **275**, 11451–11458.

van Ree, R., Fernandez-Rivas, M., Cuevas, M., van Wijngaarden, M., and Aalberse, R. (1995) Pollen-related allergy to peach and apple: an important role for profilin. *J. Allergy Clin. Immunol.*, **95**, 726–734.

Vieths, S., Frank, E., Scheurer, S., Meyer, H.E., Hrazdina, G., and Haustein, D. (1998) Characterisation of a new IgE-binding 35-kDa protein from birch pollen wuth cross-reacting homologues in various foods. *Scand. J. Immunol.*, **47**, 263–272.

Wan, L., and van Huystee, R.B. (1994) Immunogenicity of the N-glycans of peanut peroxidase. *Phytochemistry*, **37**, 933–940.

Wilson, I.B.H., and Altmann, F. (1998) Structural analysis of N-glycans from allergenic grass, ragweed and tree pollens: Core α1,3-linked fucose and xylose present in all pollens examined. *Glycoconj. J.*, **15**, 1055–1070.

Wilson, I.B.H., Harthill, J.E., Mullin, N.P., Ashford, D.A., and Altmann, F. (1998) Core α1,3-fucose is a key part of the epitope recognized by antibodies reacting against plant N-linked oligosaccharides and is present in a wide variety of plant extracts. *Glycobiology*, **8**, 651–661.

Zeleny, R., Altmann, F., and Praznik, W. (1997) A capillary electrophoretic study on the specificity of β- galactosidases from *Aspergillus oryzae*, *Escherichia coli*, *Streptococcus pneumoniae*, and *Canavalia ensiformis* (jack bean). *Anal. Biochem.*, **246**, 96–101.

Zeleny, R., Altmann, F., and Praznik, W. (1999) Structural characterization of the *N*-linked oligosaccharides from tomato fruit. *Phytochemistry*, **51**, 199–210.